

Translation

PATENT COOPERATION TREATY

PCT/JP2003/014028



PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PC-8973	FOR FURTHER ACTION See Form PCT/IPEA/416	
International application No. PCT/JP2003/014028	International filing date (day/month/year) 31 October 2003 (31.10.2003)	Priority date (day/month/year) 31 October 2002 (31.10.2002)
International Patent Classification (IPC) or national classification and IPC C12N 15/85, 5/10		
Applicant RIKEN		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of <u>5</u> sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
a. <input type="checkbox"/> (sent to the applicant and to the International Bureau) a total of _____ sheets, as follows:
<input type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
<input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
b. <input checked="" type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) <u>Disc 1</u> , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
<input checked="" type="checkbox"/> Box No. I Basis of the report
<input type="checkbox"/> Box No. II Priority
<input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
<input checked="" type="checkbox"/> Box No. IV Lack of unity of invention
<input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
<input type="checkbox"/> Box No. VI Certain documents cited
<input type="checkbox"/> Box No. VII Certain defects in the international application
<input type="checkbox"/> Box No. VIII Certain observations on the international application

Date of submission of the demand 12 July 2004 (12.07.2004)	Date of completion of this report 20 October 2004 (20.10.2004)
Name and mailing address of the IPEA/JP	Authorized officer
Facsimile No.	Telephone No.

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Box No. I Basis of the report

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

This report is based on translations from the original language into the following language _____, which is language of a translation furnished for the purpose of:

international search (under Rules 12.3 and 23.1(b))
 publication of the international application (under Rule 12.4)
 international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the elements of the international application, this report is based on (replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):

The international application as originally filed/furnished

the description:

pages _____, as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

the claims:

pages _____, as originally filed/furnished

pages* _____, as amended (together with any statement) under Article 19

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

the drawings:

pages _____, as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. The amendments have resulted in the cancellation of:

the description, pages _____
 the claims, Nos. _____
 the drawings, sheets/figs _____
 the sequence listing (specify): _____
 any table(s) related to sequence listing (specify): _____

4. This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

the description, pages _____
 the claims, Nos. _____
 the drawings, sheets/figs _____
 the sequence listing (specify): _____
 any table(s) related to sequence listing (specify): _____

* If item 4 applies, some or all of those sheets may be marked "superseded."

Box No. IV Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- restricted the claims.
- paid additional fees.
- paid additional fees under protest.
- neither restricted nor paid additional fees.

2. This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- complied with.
- not complied with for the following reasons:

The suppressor tRNA derived from eubacteria that functions in animal cells (if necessary, see: EF Wawrousek et al., Two large clusters with thirty-seven transfer RNA genes adjacent to ribosomal RNA gene sets in *Bacillus subtilis*. Sequence and organization of *trnD* and *trnE* gene clusters, *J. Biol. Chem.*, Mar 1984, Vol. 259, p. 3694-3702), which is the matter held in common by the inventions of claims 1-16, is publicly known. Therefore, the above common matter cannot be recognized as a special technical feature, and the inventions of claims 1-16 are not so linked as to form a single general inventive concept.

As a result, the inventions of claims 1-16 can be divided into the group of inventions concerning the method for expressing a protein containing an unnatural amino acid in animal cells set forth in the inventions of claims 1-13 and the group of inventions concerning recombinant DNA derived from the suppressor tRNA of *Bacillus stearothermophilus* set forth in the inventions of claims 14-16.

4. Consequently, this report has been established in respect of the following parts of the international application:

all parts.

the parts relating to claims Nos. _____ 1-13 _____

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)

Claims 1-13 YES

NO

Claims _____

Inventive step (IS)

Claims 1-13 YES

NO

Claims _____

Industrial applicability (IA)

Claims 1-13 YES

NO

Claims _____

2. Citations and explanations (Rule 70.7)

Document 1: Seikagaku, August 25, 2002, Vol. 74, No. 8, p. 1011

Document 2: Proc Natl Acad Sci USA., July 23, 2002, Vol. 99, No. 15, p. 9715-20

Based on the descriptions in documents 1 and 2, the inventions of claims 1-13 are novel and involve an inventive step.

Documents 1 and 2 describe the site-specific incorporation of iodo-tyrosine by incorporating mutant tyrosyl-tRNA synthetase from *Escherichia coli*, suppressor tRNA from *E. coli*, and iodo-tyrosine into a wheat (eucaryote) germ cell-free translation system.

Moreover, it states that this can be expected to be applicable to eucaryotic organisms in general containing this *in vivo* system.

The inventions of claims 1-13 and the inventions described in documents 1 and 2 hold in common the fact that mutant tyrosyl-tRNA synthetase from *E. coli* and a tyrosine derivative are used in the system derived from a eucaryotic cell for expressing a protein containing a site-specific mutation by an unnatural amino acid.

However, the expression system described in documents 1 and 2 differs from the inventions of claims 1-13 with respect to the fact that it uses a wheat germ cell-free translation system and suppressor tRNA from *E. coli*.

Moreover, the Specification of this application discloses that the suppressor tRNA from *E. coli* that is predicted by documents 1 and 2 to be effective in an expression system using animal cells actually does not function therein, whereas expression of a protein containing an unnatural amino acid is possible by using suppressor tRNA from *Bacillus stearothermophilus*.

As a result, documents 1 and 2 neither describe nor suggest that expression of a protein containing an unnatural amino acid is possible by combining the mutant tyrosyl-tRNA synthetase from *E. coli* and the suppressor tRNA from eubacteria such as *Bacillus stearothermophilus*, and this matter is not obvious to persons skilled in the art.

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. 1, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis that of:

a. type of material

a sequence listing
 table(s) related to the sequence listing

b. format of material

in written format
 in computer readable form

c. time of filing/furnishing

contained in the international application as filed
 filed together with the international application in computer readable form
 furnished subsequently to this Authority for the purpose of search and/or examination
 received by this Authority as an amendment* on _____

2. In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* If item 4 in Box No. 1 applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded".